Acta Protozool. (2001) 40: 291 - 296

ACTA
PROTOZOOLOGICA

Fine Structure and Biometric Characterization of the Shell in the Rare Testacean Species *Hyalosphenia punctata* Penard (Protozoa: Testacealobosia)

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Summary. *Hyalosphenia punctata* Penard was described from the sediment of Swiss lakes. Following its recognition this species was only sparsely detected, especially out of the area of its description. Because of its rare occurrence fine structure of its shell has been unknown. The present study gives new insights into the shell composition of *H. punctata* on the occasion of its occurrence in the Hungarian section of the River Danube and suggests a change of its generic position in the future, possibly into genus *Nebela*. Biometric characterization of the shells from different populations shows a negligible fluctuation within the range of the shell size.

Key words: biometric characterization, fine structure, *Hyalosphenia*, morphology, taxonomy, testate amoebae.

INTRODUCTION

The majority of testate amoeba species can be identified exclusively on the basis of the shell morphology. However, in certain species some morphological details were overseen or failed to be concerned as a determining trait at the time of the original description. The improved microscopic techniques provide the opportunity to reveal some new peculiarities in the fine structure of such species and it becomes possible to add them to the morphological description of the species.

According to composition, main types of the shell in testate amoebae are proteinaceous, agglutinate, siliceous and calcareous. Besides composing material there is an organic matrix that plays a determining role: it either forms a continuous sheet constructing the whole shell (in which the shell material may be embedded), or represents merely a binding material, which connects the building units (Ogden 1990).

The genus *Hyalosphenia* Stein, 1857 is characterized by a proteinaceous shell, where organic cement forms a continuous sheet, without external elements.

The aquatic testacean species *Hyalosphenia punctata* Penard, 1891 has turned up quite rarely since its description, only few papers report its appearance (Bhatia, Forel, Günthert, Monti; see Grospietsch 1965; Opravilova 1974, 1980). It is said to prefer the deep water zone of cold, alpine lakes (Penard 1891, 1902; Grospietsch 1965). However, it has also been found in a swamp (Penard 1902) and running waters (Opravilova

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1974, 1980). I have also encountered this species in lotic environment, during a survey of benthic testaceans in the main channel of the Szigetköz-section of the River Danube. Since three distinct samples harboured considerable numbers of specimens, it became possible to examine, whether they represent the same population or not.

My microscopic observations revealed that this species has a unique shell structure within the genus, which has been referred to as "punctuated" (see species name).

Since this rare species is very poorly documented in the scientific literature, it seemed to be reasonable to publish these new observations on this species, supplemented with taxonomic remarks and a biometric characterization of the shell.

MATERIALS AND METHODS

Hyalosphenia punctata Penard, 1891 was found in core samples, collected from the sediment of the main channel of the Szigetköz-Danube within the frame of a three-year study on benthic protozoans of the River Danube carried out between 1995-98. For sampling details see Török (1997). *H. punctata* was found in the sediment samples originating from the 1812, 1813, 1828, 1843 and 1846 river km. Table 1 shows the characteristics of the samples comprising considerable numbers of specimens.

Sediment samples were fixed and stained with Bereczky's method (Bereczky 1985).

First the tests were investigated under light microscope using bright field, phase and Nomarski optics. The first observations were made on the wet material and afterward certain tests were embedded into Euparal as permanent slides. Since this species has an extraordinary fragile shell, it has always collapsed or lost its original form when exposed to air. To avoid this, the light micrographs of *H. punctata* were made on wet material.

For scanning electron microscopic (SEM) study shells were air dried, then coated with gold. SEM investigation was made with a HITACHI S-2360N machine operating at 9 kV electron beam acceleration voltage.

Measurements and biometric characterization were made according to Schönborn *et al.* (1983).

RESULTS

The shell was transparent, made up from small, nearly circular building units of *ca* 1µm diameter each, which did not seem to overlap (Figs 1-9). Using the highest light microscopic magnification, they could be seen next to the pseudostome as well (Figs 6, 9). The shell structure seemed to fade toward the pseudostome. There was a narrow lip around the pseudostome (Figs 1, 4, 6). The coloration of the detected specimens varied slightly: one specimen showed a yellowish hue throughout the whole test, while another one was yellow at the aboral and the middle part and colourless near the pseudostome. In some of the cells the plasma filled up almost the whole shell. No epipodia were found (Figs 10-11). Food content consisted of bacteria, and algae, especially diatoms (Figs 10-12). A single nucleus with a large globular nucleolus was clearly visible in some of the cells (Figs 13, 14).

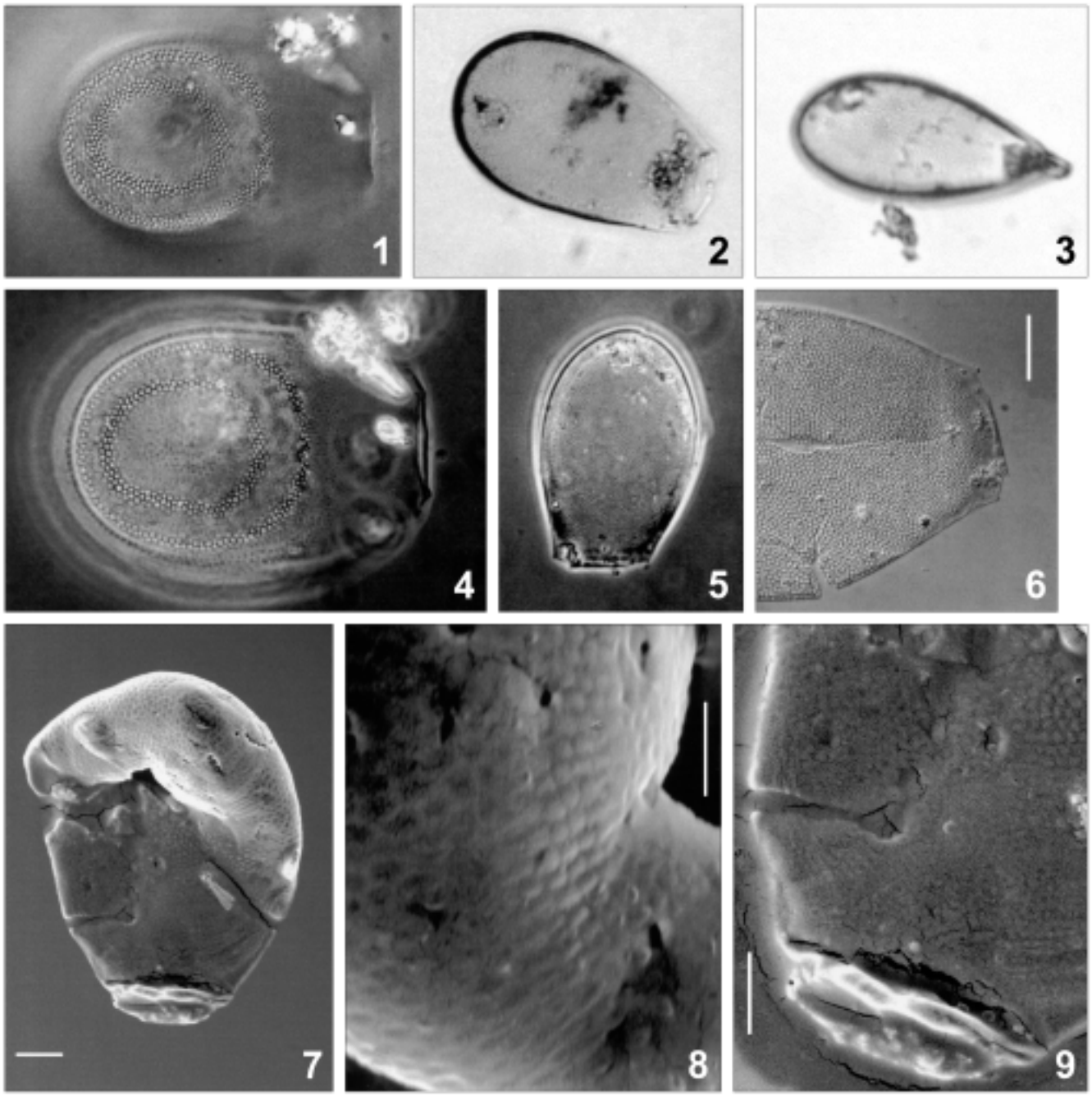
Biometric characterization of 51 shells was carried out. The values are represented together with those measured by other authors (Table 2). To compare the shell parameters in the three populations with considerable number of specimens to the rest of the shells I carried out their separate morphometric analyses (Table 3).

DISCUSSION

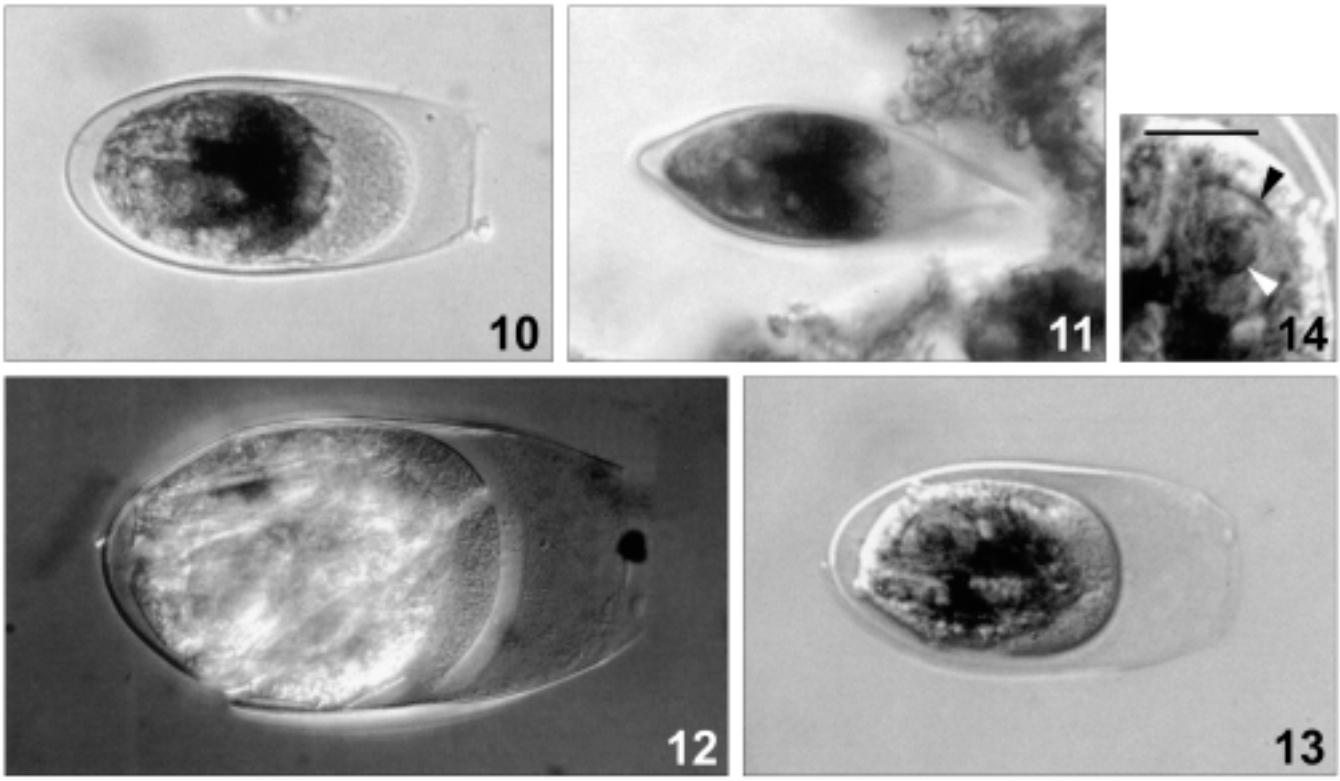
Morphology

Besides its rare occurrence, the lack of data on *H. punctata* fine structure may be attributed to its fragile test which is seemingly structureless, hyalin at low magnification. Moreover, it might get damaged or disappear in course of any kinds of manipulation (e.g. flotation, filtration) of the sample. Owing to its transparency and relatively small size, it is easy to oversee the shells in a turbid sediment sample. In the scanning electron micrographs the building units at the aboral part were much better visible than those toward the pseudostome, suggesting that the units are coated with an outer homogeneous organic layer which is getting more pronounced toward the adoral part (Figs 7-9).

The unique composition of the shell reminds me of *Arcella* shells, which are composed of proteinaceous building units bound together with organic cement, resulting a honeycomb pattern and deposit iron progressively with age. A thin outer and inner cement sheet covers the joined units (Netzel 1975, 1977). In *H. punctata*, in higher magnification building units and their arrangement are very similar to those of *Arcella* species, since both light and SEM micrographs suggest that the surface of the shell is covered with an outer - presumably organic - layer, especially at the adoral part, thus fading the underlying shell structure. Transmission electron micrographs would settle the question.



Figs 1-9. Empty shells of *Hyalosphenia punctata*. **1** - aboral punctuation of the shell, frontal view, length 75 µm (Nomarsky differential interference contrast); **2** - frontal view of another specimen, length 75 µm (bright field); **3** - lateral view of the shell in Fig. 1 (bright field); **4** - same specimen as in Figs 1, 3 with emphasized pseudostome (phase contrast); **5, 6** - frontal view of another specimen, length 92 µm (phase contrast), with detailed adoral shell structure, scale bar - 20 µm (Nomarsky differential interference contrast); **7-9** - frontal view showing the building units, with details of the aboral and adoral parts, scale bars - 10 µm, 5 µm, 7 µm, respectively (scanning electron micrographs)



Figs 10-14. Full shells of *Hyalosphenia punctata*. **10, 11** - frontal and lateral view of a specimen fixed and stained alive, length 74 µm (bright field); **12** - specimen with ingested diatoms, length 71µm (bright field); **13** - specimen with clearly visible nucleus, length 63 µm (bright field); **14** - detail of Fig.13, showing nucleolar envelope (black arrowhead) and single globular nucleolus (white arrowhead), scale bar - 10 µm (bright field)

Table 1. Samples inhabited by populations with a considerable number of specimens of *Hyalosphenia punctata*

Sample	Location	Date of collection	Microhabitat	Living specimens (%)
RAo/10/96	1812 river km, near the right side bank	October, 1996	surface of the sediment	55.5
LAo/10/96	1813 river km, near the left side bank	October, 1996	surface of the sediment	5
LAo/10/97	1813 river km, near the left side bank	October, 1997	surface of the sediment	100

Cyphoderia species with the same size of building units possess siliceous scales arranged in more regular rows, not similar to the structure of *H. punctata*. Penard (1902) has demonstrated that the scales *H. punctata* are insoluble in sulphuric acid and concluded that they are siliceous. An X-ray microanalysis in the future could prove this observation.

My measurements of the shells (Table 2) correspond well with the values given in the literature, although the references on the shell size are restricted only to the shell length (Penard 1902, Opravilova 1974). Majority of the specimens I saw were originating from three distinct samples. The separate morphometric analysis of the three samples with living specimens resulted quite homogeneous median values for the main classical features (length, breadth of the shell and shell breadth over the aperture) (Table 3). Populations 2 and 3, the richest ones in specimens, resulted from a consecutive sampling from the same locality. They share almost the same median values, only the shell breadth over the pseudostome varies slightly. This fact suggests that the two populations are identical, i.e. *H. punctata* survives there over

Table 2. Biometric characterization of *Hyalosphenia punctata* Penard, based on pooled data of the present study, including measurements from other experts to compare. ($\bar{x}$ - arithmetic mean, M - median, s - standard deviation, $s_{\bar{x}}$ - deviation of the arithmetic mean, CV - coefficient of variation, Min - minimum, Max - maximum, N - number of specimens, Ps denotes the breadth of shell over the pseudostome, shell measurements are in μm)

	$\bar{x}$	M	s	$s_{\bar{x}}$	CV	Min	Max	N
Length								
Penard	-	-	-	-	-	55	90	-
Opravilova	-	-	-	-	-	69	93	8
Present work	75.54	75	9.42	6.14	12.5	35	95	51
Breadth	38.97	40	7.14	4.31	18.3	16	54	47
Depth	26.75	25	4.05	2.75	15.1	25	35	6
Ps	25.15	25	3.01	2.49	11.9	15	32	44

Table 3. Separate biometric characterization of three populations and randomly found specimens of *Hyalosphenia punctata* Penard detected in course of the present work. (P1 - population from the 1812 river km, sample RAo/10/96; P2 - population from the 1813 river km, sample LAo/10/96; P3 - population from the 1813 river km, sample LAo/10/97; Rs - randomly found specimens from all localities. Values of the three populations are also included in group Rs, as specimens, represented by their median values). For further explanation see Table 2

	$\bar{x}$	M	s	$s_{\bar{x}}$	CV	Min	Max	N
P1								
Length	82	82.5	7.18	5.56	8.76	73	95	9
Breadth	40.11	40	5.05	3.37	12.59	32.5	51	9
Ps	24.75	25	2.49	1.56	10.06	22	30	8
P2								
Length	76.97	75	5.11	3.92	6.64	70	90	19
Breadth	39.36	40	3.83	2.43	9.73	35	52	18
Depth	25.12	25	0.25	0.19	0.99	25	25.5	4
Ps	24.91	25	2.15	1.38	8.63	20	27.5	17
P3								
Length	76.38	75	5.14	4.49	6.73	70	85	13
Breadth	39.21	40	2.59	2.06	6.60	34.5	42.5	12
Ps	26.25	26	2.14	1.62	8.15	22	30	12
Rs								
Length	68.81	75	14.35	11.08	20.85	35	85	13
Breadth	39.88	40	8.99	5.19	22.55	16	54	12
Ps	24.50	25	6.08	4.20	24.81	15	37	10

longer periods of time. Population 1 has somewhat larger length dimensions.

Ecology and distribution

Although *Hyalosphenia punctata* was listed already in Penard's first large monograph on the Swiss lakes (Penard 1891), most information referring to this species still originate from the same author (Penard 1891, 1902). Some more, scattered references are also available from other experts, but all of them are confined to establish the presence of the species (Bhatia, Forel, Güntert, Monti; see Grospietsch 1965). Opravilova published some size and ecological data (1974, 1980).

In the Danube sediment *H. punctata* was found only occasionally, which suggests its low individual abundance. This is not astonishing under the severe conditions reinforced by the irregular water regime of this river section. However, the longer survival of the same testacean population (P2 and P3 in Table 3) at the same geographic locality is of great significance. Low abundance with either very few detected specimens (8) or low dominance and frequency values was also found by

Opravilova (1974, 1980). These support the author's view that it is reasonable to regard *H. punctata* as a rare testate amoeba species.

Regarding its typical habitat, *H. punctata* was considered among the typical inhabitants of the deep-water benthos of cold, alpine lakes (Penard 1902, Grospietsch 1965). However, the specimens in the studies of Opravilova and the present study were found in running waters. Bityska Brook and Bobrav Stream are small streams with a maximum of 0.3 and 1.5 m depth, and 5 and 10 m width, respectively (Opravilova 1974, 1980). On the contrary, the sampled region of the Danube has about three - four hundred m^3/s mean water discharge. This is with more than one order of magnitude larger than the two streams studied by Opravilova. The found specimens can either be accidental elements in the local species assemblages of the lotic environment or can originate from the lentic habitats of the extended side arm systems of the Danube (Szigetköz and Csallóköz). It is regrettable that there was no reference to the exact microhabitat type where *H. punctata* occurred in the Bobrav Stream (Opravilova 1974). In the Bityska Brook *H. punctata* was found exclusively in one sampling locality, the sediment (Opravilova 1980).

Reflections on taxonomy

The revealed particular shell structure of *H. punctata* makes questionable the present generic position of the species since the revealed structure conflicts with the general definition of the genus which declares that shells are composed of homogeneous proteinaceous material. The general form of the shell and the rim around the pseudostome are arguments to the advantage of *Hyalospheniidae* Schulze, 1877. However, the observed composition of the shell suggests that this species might be rather a member of genus *Nebela* than a *Hyalosphenia*. Further observations, including more living specimens and recognition of the origin and chemical nature of the composing shell units might result in placing *H. punctata* into the genus *Nebela*. But, before making this change, the Filosean origin of the building units has to be proved.

Acknowledgements. I am very grateful to Dr. Z. Kristóf for making the SEM micrographs, Mr. M. Balogh for his help with the SEM preparation and Dr. M. Cs. Bereczky for comments on the manuscript. This study was financed by the Hungarian Scientific Research Foundation (OTKA, T-17708).

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Received on 8th May, 2001; accepted on 4th September, 2001